(f) *Calculations.* (1) Calculate the micrograms of cefuroxime per milligram of sample as follows:

$$\text{Micrograms of cefuroxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) Calculate the cefuroxime content of the vial as follows:

$$\text{Milligrams of cefuroxime per vial} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter; and

d= Dilution factor of the sample.

[48 FR 38460, Aug. 24, 1983; 48 FR 40704, Sept. 9, 1983]

§ 436.344 Thin layer chromatographic identity test for cefuroxime.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23×23×9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover. Line the inside walls of the tank with Whatman #3MM chromatographic paper or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel F or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix chloroform, methanol, and formic acid in volumetric proportions of 90:16:4, respectively.

(c) *Preparation of the spotting solutions.* Dissolve approximately 200 milligrams each of the working standard and sample in 5 milliliters of a 50 percent aqueous acetone solution.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot 5 microliters each of the sample and working standard solutions. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel a minimum of 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry. Observe under ultraviolet light (254 nanometers).

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

[48 FR 38461, Aug. 24, 1983]

§ 436.345 High-pressure liquid chromatographic assay for ceftizoxime.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *pH 3.6 buffer solution.* Transfer 2.31 grams of sodium phosphate diabasic dodecahydrate and 1.42 grams of citric acid monohydrate to a

1-liter volumetric flask. Dissolve and dilute to volume with distilled water.

(2) *pH 7.0 buffer solution.* Transfer 14.33 grams of sodium phosphate dibasic dodecahydrate and 3.63 grams of potassium phosphate monobasic to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water.

(3) *Mobile phase.* Mix pH 3.6 buffer solution:acetonitrile (9:1). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(4) *Internal standard solution.* Place 1.2 grams of salicyclic acid in a 200-milliliter volumetric flask. Dissolve in 10 milliliters of methyl alcohol, dilute to volume with pH 7.0 buffer solution and mix.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the ceftizoxime working standard with sufficient pH 7.0 buffer solution to obtain a solution containing 1,000 micrograms of ceftizoxime activity per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix.

(e) *Preparation of sample solutions*—(1) *Product not packaged for dispensing (micrograms of ceftizoxime per milligram).* Dissolve an accurately weighed portion of the sample with sufficient pH 7.0 buffer solution to obtain a concentration of 1.0 milligram per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix. Using this sample solution, proceed as directed in paragraph (f) of this section.

(2) *Product packaged for dispensing.* Determine both micrograms of ceftizoxime per milligram of the sample and milligrams of ceftizoxime per container. Use separate containers for preparation of each sample solution as described in paragraphs (e)(2) (i) and (ii) of this section.

(i) *Micrograms of ceftizoxime per milligram.* Dissolve an accurately weighed portion of the sample with sufficient pH 7.0 buffer solution to obtain a concentration of 1.0 milligram of ceftizoxime per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix. Using this sample solution, proceed as directed in paragraph (f) of this section.

(ii) *Milligrams of ceftizoxime per container.* Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency is a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of the solution thus obtained with sufficient pH 7.0 buffer solution to obtain a concentration of 1.0 milligram per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix. Using this sample solution, proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. The elution order is void volume, ceftizoxime, and internal standard. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (e)(1) of this section into the chromatograph and repeat the procedure described for the working standard solution. If the sample is packaged for

dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (e)(2) (i) and (ii) of this section.

(g) *Calculations*—(1) Calculate the micrograms of ceftizoxime per milligram of sample as follows:

$$\text{Micrograms of ceftizoxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u=Area of the ceftizoxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the ceftizoxime peak in the chromatogram of the ceftizoxime working standard/Area of internal standard peak;

P_s=Ceftizoxime activity in the ceftizoxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

$$\text{Milligrams of ceftizoxime per vial} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the ceftizoxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the ceftizoxime peak in the chromatogram of the ceftizoxime working standard/Area of internal standard peak;

P_s=Ceftizoxime activity in the ceftizoxime working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

[48 FR 46270, Oct. 12, 1983; 48 FR 49656, Oct. 27, 1983]

§ 436.346 High-pressure liquid chromatographic assay for cyclosporine.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A suitable pump capable of reproducibly delivering a liquid to a pressure of 4,500 pounds per square inch and a flow rate of at least 5 milliliters per minute;

(2) A suitable ultraviolet detection system operating at a wavelength of 210 nanometers;

(3) A suitable recorder;

(4) A suitable integrator;

(5) An oven or water bath capable of maintaining the column at an operating temperature of 70° C;

(6) A steel capillary tube, 1 meter in length, having an inside diameter of 0.25 millimeter. This tube is inserted between the injection system and the chromatographic column and is equilibrated to 70° C; and

(7) A sample injection valve on which the loop determines the sample size.

(b) *Columns.* The chromatographic column is packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing materials that exhibit some degree of polarity such as the hydrocarbon bonded silicas with dimethyl, trimethyl, or octyl groups. Connect a saturation column gravity packed with similarly bonded silica particles 40 to 60 microns in diameter to the inlet of the analytical column.

(c) *Mobile phase.* Mix acetonitrile, water, methanol, and *o*-phosphoric acid (900:525:75:0.075 by volume). Degas by passing through a 0.5-micrometer filter with vacuum and ultrasonicate for no less than 2 minutes before use. The mobile phase may be sparged perceptibly with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of acetonitrile to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(d) *Operating conditions.* Perform the assay at a constant operating temperature of 70° C with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with a typical chart speed of 2.5 millimeters per minute. Obtain chromatograms for performance parameters at a chart speed of not less than 25 millimeters per minute to allow a more accurate measurement of peak geometry.

(e) *Preparation of working standard and sample solutions.* Prepare the working standard and sample solutions as directed in the individual monographs for cyclosporine.

(f) *Systems suitability.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by about 5 injections of not less than 10 microliters each of working standard solution. Proceed